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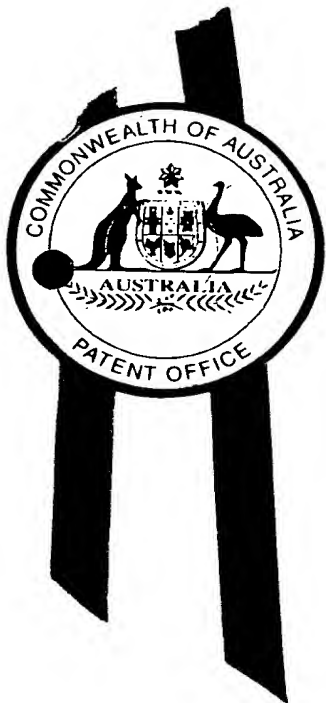
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I, KAY WARD, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PQ 0652 for a patent by UNIVERSITY OF SYDNEY filed on 28 May 1999.

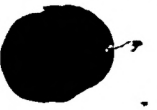
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KAY WARD  
TEAM LEADER EXAMINATION  
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University of Sydney

**A U S T R A L I A**

**Patents Act 1990**

**PROVISIONAL SPECIFICATION**

for the invention entitled:

"A method of prophylaxis and treatment and agents useful for same"

The invention is described in the following statement:

- 1A -

## A METHOD OF PROPHYLAXIS AND TREATMENT AND AGENTS USEFUL FOR SAME

The present invention relates generally to a method for the prophylaxis and treatment  
5 of infection by microorganisms in biological environments from where the  
microorganisms acquire iron, heme or porphyrin, generally but not exclusively for  
growth. Particular biological environments contemplated by the present invention  
include but are not limited to vascular regions and cavities as well as mucosal  
membranes in animals including mammals, reptiles, amphibians and fish and in avian  
10 species as well as hooves of livestock animals. The method of the present invention  
involves interrupting, reducing or otherwise antagonising the interaction between a  
microbial-derived polypeptide, such as but not limited to a polypeptide having cysteine  
proteinase activity, and a porphyrin-containing molecule in such as heme. The  
present invention further provides agents useful in the prophylaxis and treatment of  
15 microbial infection of biological environments such as vascular regions and cavities  
including mucosal membranes as well as hooves involving microbial acquisition of  
iron, heme or porphyrin. Such agents are particularly useful as components in  
therapeutic compositions. Particularly important microbial infections targeted by the  
present invention involve infections in the oral cavity, nasopharynx, oropharynx,  
20 vagina and urethra in mammals such as humans. Other important microbial infections  
including infections of hooves in livestock animals such as sheep, cattle and goats.

Throughout this specification, unless the context requires otherwise, the word  
"comprise", or variations such as "comprises" or "comprising", will be understood to  
25 imply the inclusion of a stated element or integer or group of elements or integers but  
not the exclusion of any other element or integer or group of elements or integers.

Bibliographic details of the publications numerically referred to in this specification are  
collected at the end of the description.

30

The subject specification contains nucleotide and amino acid sequence information

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prepared using the programme PatentIn Version 2.0, presented herein after the bibliography. Each nucleotide or amino acid sequence is identified in the sequence listing by the numeric indicator <210> followed by the sequence identifier (e.g. <210>1, <210>2, etc). The length, type of sequence (DNA, protein (PRT), etc) and  
 5 source organism for each nucleotide or amino acid sequence are indicated by information provided in the numeric indicator fields <211>, <212> and <213>, respectively. Nucleotide and amino acid sequences referred to in the specification are defined by the information provided in numeric indicator field <400> followed by the sequence identifier (eg. <400>1, <400>2, etc).

10

Proteinases are enzymes which hydrolyse peptide bonds in peptides, polypeptides and proteins. One particular group of proteinases, the endopeptidases, cleave bonds within the peptide chain with varying degrees of specificity for particular amino acyl residues. An example of an endopeptidase is serine proteinase which is characterized  
 15 by a catalytically active serine residue in its active centre. Another example is a cysteine proteinase (sometimes referred to as a thiol proteinase) which has free -SH groups in its active centre.

There is increasing evidence for the potential importance of proteinases in microbial  
 20 infection. This is particularly highlighted by the involvement of cysteine proteinases in periodontal disease pathology caused by the Gram negative microorganism, *Porphyromonas gingivalis*. This microorganism was formally known as *Bacteroides* sp.

Periodontal disease affects a majority of adults in varying degrees and is associated  
 25 with significant systemic morbidity (1, 1a) including dental infection and loss of teeth. *Porphyromonas gingivalis* is implicated as an important pathogen by its high incidence and relative levels in human disease (2, 2a) and by its virulence in mono-infected animals (3, 4). Virulence of *P. gingivalis* has been attributed to several components of the microorganism including fimbriae (5, 6), short-chain volatile acids (7, 8),  
 30 lipopolysaccharide (9, 10), collagenase activity (11, 12) and non-collagenolytic cysteine proteinase activity (13, 14, 15).

Cysteine proteinases have a range of activities including affecting the remodelling of matrix proteins and disrupting the immune response by stimulating collagen-degrading activity of host cells (13, 14, 16), degrading fibronectin (17), inactivating interferon- $\alpha$  (19) and interleukins (18, 20), interfering with the complement cascade (21, 22) and  
 5 degrading immunoglobulins (23, 24). Furthermore, clotting and vascular permeability mechanisms may be disturbed (15, 25, 26), fibrinogen may be degraded (15, 27) and erythrocytes agglutinated and lysed (28, 29) by cysteine proteinase activity.

A number of *P. gingivalis* cysteine proteinases described in several reports have been  
 10 demonstrated to be antigenically related (14, 30, 31) and the products of three related genes (32, 33). Cysteine proteinases from *P. gingivalis* are generally referred to as gingipains. Two major gingipains, Arg-gingipain-1 (RGI-1) and Lys-gingipain (KGP) [32], prefer proteinaceous substrates with an arginine or lysine in the P1 position, respectively.

15

The gingipains are expressed on the outer membrane of *P. gingivalis* and may also be released with residues or as soluble proteins (34, 35, 36). It has been proposed that *P. gingivalis* binds to hemoglobin via the gingipains (38).

20 The catalytic domains of RGP-1 and KPG constitute approximately one third of the translated protein product. C-terminal to the catalytic domain, there are the following four domains: HA1, HA2, HA3 AND HA4 which are highly homologous between RGP-1 and KPG. These non-catalytic COOH-terminal domains have previously been named hemagglutinin (HA) domains because at least one was thought to participate in  
 25 hemagglutination (30). Because all of the domains of the gingipains are found together predominately in loose, non-covalent associations with one another after hydrolytic separation (34, 37), the gingipains appear to be multifunctional proteins for aggregating erythrocytes then lysing these cells to obtain hemoglobin for the acquisition of iron, heme and/or porphyrin.

30

The elucidation of the molecular and biochemical mechanisms involved in key

regulatory pathways, such as pathways involving the acquisition of iron, heme and porphyrin, is paramount in developing strategies for the control of disease. The inventors have now determined the molecular mechanism of HA2 domain binding to porphyrin-containing molecules such as hemoglobin and in particular heme. The  
5 elucidation of the mechanisms underlying hemoglobin binding provides a means for the rational design of antagonists to prevent, reduce or otherwise retard the growth and maintenance of microorganisms which require exogenous iron, heme or porphyrin.

Accordingly, one aspect of the present invention contemplates a method for the  
10 prophylaxis or treatment of infection by a microorganism in a biological environment from where the microorganism acquires iron, heme or porphyrin said method comprising administering to said environment an effective amount of an agent for a time and under conditions sufficient to antagonise the interaction between a molecule derived from said microorganism and having an HA2 domain and an HA2-binding motif  
15 on a porphyrin-containing molecule present in said biological environment.

The term "biological environment" is used in its broadest context to include an environment comprising porphyrin-containing molecules. Particularly preferred porphyrin-containing molecules include hemoglobin and its precursors as well as  
20 heme. Preferably, the biological environment is a vascular region or cavity or a mucosal membrane in an animal species such as a mammal, reptile, amphibian, fish or bird or is a hoof of a livestock animal. More preferably, the animal is a mammal such as a human or livestock animal.

25 Accordingly, a preferred aspect of the present invention provides a method for the prophylaxis or treatment of infection by a microorganism in a mammal from where the microorganism acquires iron, heme or porphyrin said method comprising administering to said environment an effective amount of an agent for a time and under conditions sufficient to antagonise the interaction between a molecule derived from said  
30 microorganism and having an HA2 domain and an HA2-binding motif on a porphyrin-containing molecule such as but not limited to hemoglobin or a precursor

form thereof or part thereof such as heme.

Although the present invention is particularly directed to *P. gingivalis* infection in the oral cavity such as during periodontal disease, it extends to any disease condition  
 5 resulting from microbial infection and in particular infection by *P. gingivalis* or a related microorganism involving the acquisition of iron, heme or porphyrin. Such microorganisms are required to acquire iron, heme or porphyrin as they do not possess a biosynthetic pathway for porphyrins. Examples of microorganisms related to *P. gingivalis* contemplated herein include but are not limited to *Salmonella sp.*,  
 10 *Serratia sp.*, *Yersinia sp.*, *Klebsiella sp.*, *Vibrio sp.*, *Pseudomonas sp.*, *E. coli*, *Haemophilus sp.* and *Bordetella sp.* Examples of *P. gingivalis* or related microorganism infection contemplated by the present invention include infection of the oral cavity, nasopharynx, oropharynx, vagina and urethra as well as infection of mucosal membranes and infection of hooves of livestock animals such as sheep, cattle and  
 15 goats. An "effective" amount means a porphyrin-binding interfering effective amount, i.e. an amount sufficient to interfere with HA2 domain interaction with a porphyrin moiety.

Another aspect of the present invention provides a method for the prophylaxis or  
 20 treatment of infection by a microorganism in a mammal, said microorganism being substantially incapable of synthesizing porphyrins said method comprising administering to said mammal an effective amount of an agent for a time and under conditions sufficient to antagonise the interaction between a molecule derived from said microorganism and having an HA2 domain and an HA2-binding motif on a  
 25 porphyrin-containing molecule such as but not limited to hemoglobin or a precursor form thereof or part thereof such as heme.

A related aspect of the present invention contemplates a method for prophylaxis or treatment of periodontal, pulmonary, vaginal, urethral or hoof disease resulting from  
 30 infection by *P. gingivalis* or related microorganism in a mammal said method comprising administering to said mammal an effective amount of an agent for a time



and under conditions sufficient to antagonise the interaction between a *P. gingivalis*-derived molecule having an HA2 domain and an HA2-binding motif on a porphyrin-containing molecule such as but not limited to hemoglobin or a precursor form thereof or part thereof such as heme.

5

Reference herein to "*Porphyromonas gingivalis*" or its abbreviation "*P. gingivalis*" includes reference to all mutants, derivatives and variants of this organism as well as serological sub-types. The present invention further extends to microorganisms related to *P. gingivalis* at the metabolic, structural, biochemical, immunological and/or

10 disease causing levels. Examples of related microorganisms are those listed above.

The term "HA2" domain is used in its broadest context and includes regions having structural or functional homology to the HA2 region. An HA2 domain comprises a sequence of amino acids having conformationally and/or linearly defined binding

15 capacity to an HA2-binding motif on a porphyrin containing molecule such as hemoglobin and more particularly heme.

A particularly preferred HA2 domain comprises the following amino acid sequence:

20     Ala Asp Phe Thr Glu Thr Phe Glu Ser Ser Thr His Gly Glu Ala Pro  
       Ala Glu Trp Thr Thr Ile<sup>-</sup> Asp Ala Asp Gly Asp Gly Glu Gly Trp Leu  
       Cys Leu Ser Ser Gly Gln Leu Asp Trp Leu Thr Ala His Gly Gly Thr  
       Asn Val Val Ser Ser Phe Ser Trp Asn Gly Met Ala Leu Asn Pro Asp  
       Asn Tyr Leu Ile Ser Lys Asp Val Thr Gly Ala Thr Lys Val Lys Tyr  
   25     Tyr Tyr Pro Val Asn Asp Gly Phe Pro Gly Asp His Tyr Ala Val Met  
       Ile Ser Lys Thr Gly Thr Asn Ala Gly Asp Phe Thr Val Val Phe Glu  
       Glu Thr Pro Asn Gly Ile Asn Lys Gly Gly Ala Arg Phe Gly Leu Ser  
       Thr Glu Ala Asn Gly Ala

30 or a sequence having at least about 50% similarity to at least about 10 contiguous amino acids thereof or at least about 25% identity after optimum alignment with the same sequence. Alternative percentage similarities include at least about 60% or 70% or 80% or 90% or above. Alternative percentage identities include at least about 30%, 40%, 50%, 60%, 70%, 80% or 90% or above. An HA2 domain is also conveniently

defined by being encoded by a sequence of nucleotides comprising the following sequence:

```

5   gca gac ttc acg gaa acg ttc gag tct tct act cat gga gag gca cca
    gcg gaa tgg act act atc gat gcc gat ggc gat ggt gag ggt tgg ctc
    tgt ctg tct tcc gga caa ttg gac tgg ctc aca gct cat ggc ggc acc
    aac gta gta agc tct ttc tca tgg aat gga atg gct ttg aat cct gat
    aac tat ctc atc tca aag gat gtt aca ggc gca acg aag gta aag tac
10  tac tat cca gtc aac gac ggt ttt ccc ggg gat cac tat gcg gtg atg
    atc tcc aag acg ggc acg aac gcc gga gac ttc acg gtt gtt ttc gaa
    gaa acg cct aac gga ata aat aag ggc gga gca aga ttc ggt ctt tcc
    acg gaa gcc aat ggc gcc

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15

or a nucleotide sequence having at least 50% similarity to at least about 30 contiguous nucleotides thereof or a nucleotide sequence capable of hybridizing thereto under low stringency conditions. Alternative percentage similarities include at least about 60%, 70%, 80% or 90% or above.

20

Accordingly, another aspect of the present invention contemplates a method for the prophylaxis or treatment of infection by a microorganism in a mammal, said microorganism substantially requiring exogenous iron, heme or porphyrin for growth or maintenance wherein said method comprises administering to said mammal an effective amount of an agent for a time and under conditions sufficient to antagonise the interaction between a molecule derived from said microorganism and having an HA2 domain and an HA2-binding moiety on a porphyrin-containing molecule such as but not limited to hemoglobin or a precursor form thereof or part thereof such as heme and wherein said HA2 domain comprises:

30

(i) an amino acid sequence substantially encoded by the nucleotide sequence set forth in <400>5 or a nucleotide sequence having at least about 50% similarity thereto or capable of hybridizing thereto under low stringency conditions; and/or

- (ii) an amino acid sequence substantially as set forth in <400>6 or an amino acid sequence having at least about 50% similarity thereto or at least about 25% identity after optimum alignment with the same sequence;
- 5 wherein said amino acid sequence is capable of interacting with an HA2-binding motif on a porphyrin-containing molecule such as but not limited to hemoglobin or a precursor form thereof or part thereof such as heme.

Preferred molecules having an HA2 domain include cysteine proteinases such as  
 10 gingipains, a product of the *hagA* gene and any TonB dependent protein carrying an HA2 domain and in particular those TonB dependent proteins involved in the acquisition of heme. An example of a TonB-dependent protein is Tla (TonB-linked adhesion) [44]. The present invention, however, extends to all HA2-containing molecules.

15

The term "infection" is used in its most general sense and includes the presence or growth of *P. gingivalis* or related microorganism resulting in a disease condition or having the capacity to result in a disease condition. The term "infection" further encompasses *P. gingivalis* or related microorganism when present as part of the  
 20 normal flora. Such bacteria may, under certain circumstances, be responsible for disease development. Prophylaxis is contemplated in accordance with the present invention to reduce the levels of *P. gingivalis* or related microorganism or to reduce the likelihood of a disease condition developing resulting from infection by *P. gingivalis* or a relative thereof.

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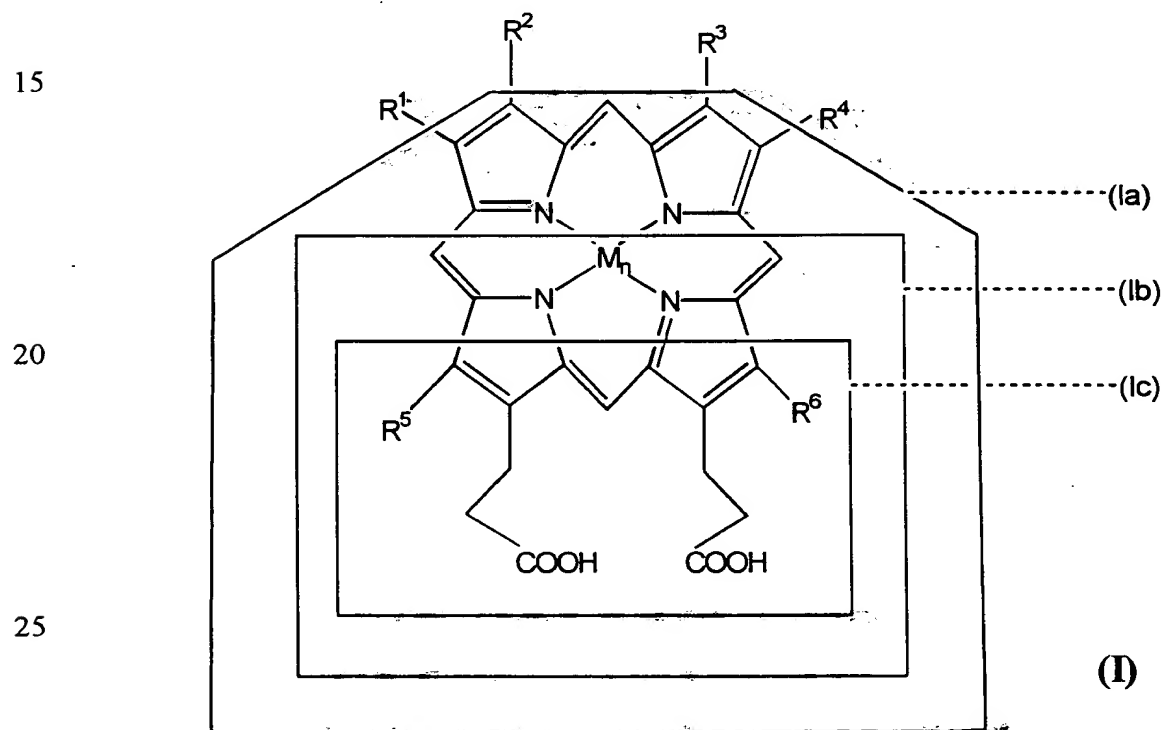
The present invention is particularly directed to the treatment of *P. gingivalis* or a related microorganism in humans. The present invention extends, however, to the prophylaxis or treatment of *P. gingivalis* or related microorganisms in other mammals such as primates, livestock animals (e.g. sheep, cows, goats, pigs, horses, donkeys),  
 30 companion animals (e.g. dogs, cats), laboratory test animals (e.g. mice, rats, guinea pigs, rabbits, hamsters) and captured wild animals.

In accordance with the present invention, it has been determined that the HA2 domain binds to a portion of the porphyrin moiety of on a porphyrin-containing molecule such as but not limited to hemoglobin or a precursor form thereof or part thereof such as heme. Preferably, the HA2 domain interacts with a surface exposed heme moiety of  
5 hemoglobin.

In a particularly preferred embodiment, the HA2 region interacts with the region on porphyrin, and in particular heme, comprising exposed propionic groups or their anionic or salt forms.

10

A porphyrin molecule is considered to have the structure shown in (I):



wherein  $R_1$  and  $R_6$  are the same or different and each is an alkyl such as a methyl, ethyl or propyl group, or hydrogen, hydroxyl, carboxyl, aldehyde, acetaldehyde or keto  
30 group,  $M$  is a metal ion in various oxidation states such as but not limited to  $Fe$ ,  $Fe^{++}$  and  $Fe^{+++}$  and is optionally present such that  $n$  is 0 or 1. A person skilled in the art will

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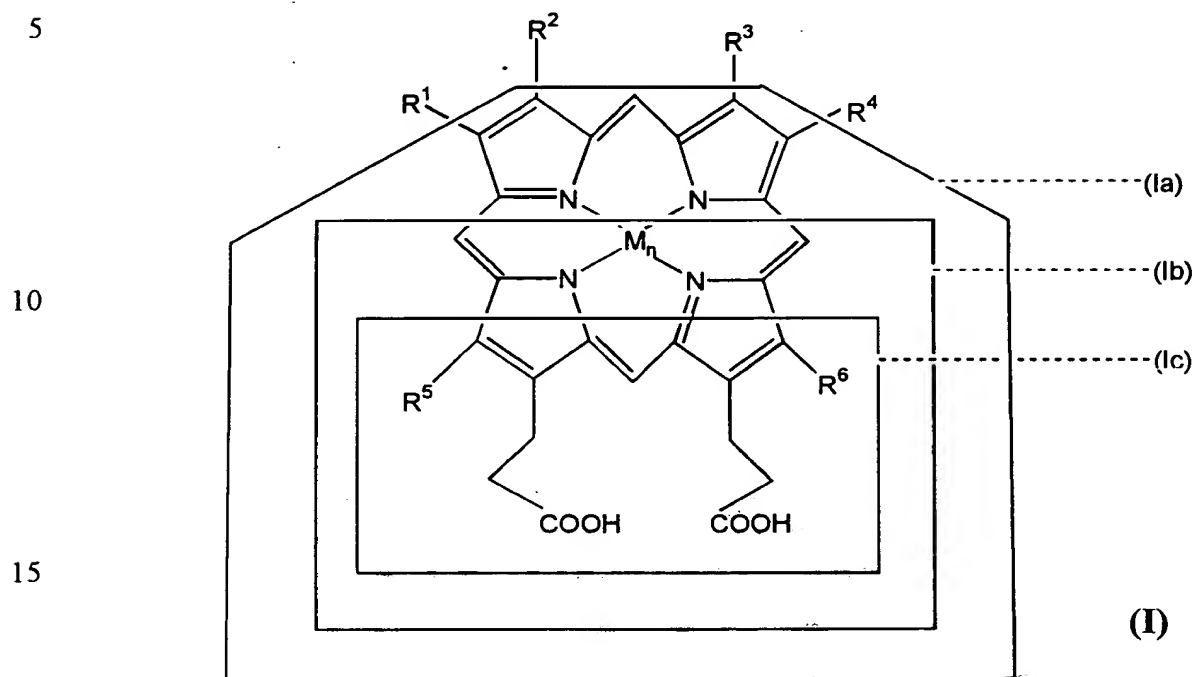
appreciate that when n is 0, the trivalency of the two  $sp^3$  hybridized N atoms will be completed by a hydrogen atom.

It is proposed in accordance with the present invention that the HA2-binding motif  
5 comprises the molecule defined by structure (I). Preferably, the HA2-binding motif  
comprises the region in substructure (1a). More preferably, the HA2-binding motif  
comprises the region in substructure (1b). Even more preferably, the HA2-binding  
motif comprises the region defined by substructure (1c). Reference herein to the  
"HA2-binding motif" includes and comprises the motif defined by substructure (1a),  
10 preferably substructure (1b) and more preferably substructure (1c) or a structurally or  
functionally homologous region capable of interacting with the HA2 domain of a  
molecule such as but not limited to a cysteine proteinase.

Accordingly, another aspect of the present invention provides a method for the  
15 prophylaxis or treatment of infection by a microorganism in a mammal, said  
microorganism substantially requiring exogenous iron, heme or porphyrin for growth  
or maintenance wherein said method comprises administering to said mammal an  
effective amount of an agent for a time and under conditions sufficient to antagonise  
the interaction between a molecule derived from said microorganism and having an  
20 HA2 domain and an HA2-binding moiety on a porphyrin-containing molecule such as  
but not limited to hemoglobin or a precursor form thereof or part thereof such as heme  
and wherein said HA2 domain comprises:

- (i) an amino acid sequence substantially encoded by the nucleotide  
25 sequence set forth in <400>5 or a nucleotide sequence having at least about 50%  
similarity thereto or capable of hybridizing thereto under low stringency conditions;  
and/or
- (ii) an amino acid sequence substantially as set forth in <400>6 or an amino  
acid sequence having at least about 50% similarity thereto or at least about 25%  
30 identity after optimum alignment with the same sequence.;

and wherein the HA2-binding motif comprises a moiety structurally or functionally homologous to substructure (1a) of structure (I) below:



Preferably, the HA2-binding motif comprises substructure (1b). More preferably, the HA2-binding molecule comprises substructure (1c).

Another aspect of the present invention contemplates a method for the prophylaxis or treatment of *P. gingivalis* infection or infection by a related microorganism in a mammal said method comprising administering to said mammal an effective amount of an agent for a time and under conditions sufficient to antagonise the interaction between a *P. gingivalis*-derived HA2-containing molecule and an HA2-binding motif on a porphyrin-containing molecule such as but not limited to hemoglobin or a precursor form thereof or part thereof such as heme.

Infection by *P. gingivalis* or related microorganism in accordance with this aspect of the present invention is one leading to or having the potential to lead to an infection

of a mucosal or vascular region such in the oral cavity, nasopharynx, oropharynx, vagina or urethra as well as the hooves of farm animals.

Reference to a "*P. gingivalis*- derived HA2-containing molecule" includes gingipains  
 5 bound to *P. gingivalis* as well as soluble forms of the cysteine proteinase and the *hagA* gene product as well as TonB-dependent proteins such as the Tla protein (44).

The term "antagonise" means and includes reducing, inhibiting or otherwise adversely affecting interaction between the HA2 domain and that part of the porphyrin ring and  
 10 in particular heme which forms the HA2-binding motif on hemoglobin. The functional result of such antagonism is the inability or at least reduced capacity of *P. gingivalis* or related microorganism for acquiring iron, heme or porphyrin for use in, for example, metabolic pathways. Antagonism may be complete, i.e. from about 90-100% or partial, i.e. from about 30 to about 90% as determined by binding assays or inhibition of *P.*  
 15 *gingivalis* growth or maintenance.

Although not intending to limit the present invention to any one theory or mode of action, it is proposed that *P. gingivalis* and its relatives do not have a complete functional porphyrin-synthesizing pathway. In particular, it is proposed that *P.*  
 20 *gingivalis* lacks one or more of a glutamyl-t RNA reductase, porphobilinogen synthase, porphobilinogen deaminase, uroporphyrinogen III cosynthase, uroporphyrinogen decarboxylase, coproporphyrinogen III oxidase, HemM or uroporphyrinogen III methylase. As a result, *P. gingivalis* needs to acquire porphyrin for growth and/or maintenance or at least to facilitate growth and/or maintenance. Accordingly, by  
 25 antagonising the interaction between the HA2 domain and the HA2-binding motif the microorganism is unable to acquire porphyrin, iron or heme and infection can be controlled.

In a particularly preferred embodiment, the antagonism results from inhibiting  
 30 interaction between a region of surface exposed porphyrin and in particular heme comprising propionic acid groups or their anionic or salt forms such as but not limited

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to the region defined by sub-structure (Ia) and an HA2 containing molecule comprising an epitope capable of interaction with monoclonal antibody mAb 5A1 (see ref 34). In accordance with the present invention, mAb 5A1 interacts with an epitope defined by amino acid sequence ALNPDNYLISKDVTG <400>1 or an amino acid sequence  
5 having at least 50% similarity thereto or at least about 25% identity after optimum alignment with same sequence including an amino acid sequence defined by <400>1 but which has single or multiple amino acid substitutions, deletions and/or additions.

The amino acid sequence defined by <400>1 is not the porphyrin binding site but a  
10 useful marker for HA2.

Accordingly, another aspect of the present invention contemplates a method for the prophylaxis or treatment of *P. gingivalis* infection or infection by a related microorganism in a mammal said method comprising administering to said mammal  
15 an effective amount of an agent for a time and under conditions sufficient to antagonise the interaction between a *P. gingivalis*-derived HA2- containing molecule comprising the amino acid sequence ALNPPNYLISKDVTG <400>1 or an amino acid sequence having at least 50% similarity to <400>1 or at least about 25% identity after optimum alignment with same sequence or an amino acid sequence encoded by the  
20 nucleotide sequence <400>7 or a nucleotide sequence having at least 50% similarity thereto or a nucleotide sequence capable of hybridizing thereto under low stringency conditions and an HA2-binding motif comprising and including propionic acid groups or anionic or salt forms thereof such as but not limited to the region defined by substructure (Ia) on a porphyrin-containing molecule such as but not limited to  
25 hemoglobin or a precursor form thereof or part thereof such as heme.

The term "similarity" as used herein includes exact identity between compared sequences at the nucleotide or amino acid level. Where there is non-identity at the nucleotide level, "similarity" includes differences between sequences which result in  
30 different amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. Where there is non-identity at



the amino acid level, "similarity" includes amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. In a particularly preferred embodiment, nucleotide and sequence comparisons are made at the level of identity rather than similarity. Alternatively, similar molecules may be  
5 determined by percentage identity (e.g. 25%) after optimum alignment with the same sequence. Preferably, the percentage identity is 25%. Any number of programs are available to compare nucleotide and amino acid sequences. Preferred programs have regard to an appropriate alignment. One such program is Gap which considers all possible alignment and gap positions and creates an alignment with the largest  
10 number of matched bases and the fewest gaps. Gap uses the alignment method of Needleman and Wunsch (43). Gap reads a scoring matrix that contains values for every possible GCG symbol match. GAP is available on ANGIS (Australian National Genomic Information Service) at website <http://mel1.angis.org.au>. Another suitable programme is BLAST.

15

Reference herein to a low stringency generally means at 42°C or within the range of from about 37°C to about 60°C and includes and encompasses from at least about 0% v/v to at least about 15% v/v formamide and from at least about 1M to at least about 2M salt for hybridisation, and at least about 1M to at least about 2M salt for washing  
20 conditions. Alternative stringency conditions may be applied where necessary, such as medium stringency, which includes and encompasses from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5M to at least about 0.9M salt for hybridisation, and at least about 0.5M to at least about 0.9M salt for washing conditions, or high stringency, which includes and encompasses from at least  
25 about 31% v/v to at least about 50% v/v formamide and from at least about 0.01M to at least about 0.15M salt for hybridisation, and at least about 0.01M to at least about 0.15M salt for washing conditions.

The identification of the molecular mechanism underlining HA2 interaction with  
30 hemoglobin provides a means for screening for antagonists of this interaction. Such antagonists are useful, for example, in the development of vaccines and therapeutic

compositions for preventing or treating infection by *P. gingivalis* or related microorganisms.

Accordingly, another aspect of the present invention provides an agent capable of  
5 antagonising interaction between an HA2-containing molecule and an HA2-binding motif on a porphyrin-containing molecule such as but not limited to hemoglobin or a precursor form thereof or part thereof such as heme.

Preferably, the agent antagonises interaction between the HA2 containing molecule  
10 and a region on a porphyrin portion of hemoglobin comprising propionic acid groups or anionic or salt forms thereof.

Preferably, the region on hemoglobin is defined by substructure (Ia) as described above.

15

The agent may be a derivative of the HA2-containing molecule (e.g. gingipain or *hagA* gene product or T1a protein) or hemoglobin or may be identified following screening of a chemical library or following natural product screening. The latter includes screening of environments such as aquatic environments, coral, seabeds,  
20 microorganisms, plants and antarctic environments for naturally occurring molecules capable of acting as antagonists.

Alternatively, the HA2-containing molecule may be crystallized and antagonists derived based on the structure of the HA2 domain.

25

Any HA2-containing molecule such as the gingipain, *hagA* gene product or T1a protein or their derivatives may be used as vaccine components to generate antibodies to their HA2 domains or their immunological relatives. Alternatively, the antagonist may be an antibody to HA2 or an antibody to another region resulting in reduced binding  
30 of the HA2-containing molecule to hemoglobin. Antibodies may be employed from any source but may need to be humanized if the intended use is in humans unless the

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antibodies are topically applied. Alternatively, the antibodies are raised by lactating dairy animals to provide passive immunization, particularly in the form of secretory antibody in dairy products.

- 5 The antagonists, therefore, may be peptides, polypeptides, proteins, antibodies, small or large chemical entities or combinations thereof and may be in isolated, naturally occurring form or may be in recombinant or chemically synthetic form.

Screening for antagonists may be accomplished in any number of ways. In one  
10 method, preparations of gingipains or HA2-containing parts thereof are incubated with potential antagonists and then subjected to chromatography or gel electrophoresis or immunoassay to screen for the formation of a complex.

In one particularly useful method, incubation mixtures of HA2-containing molecules  
15 and potential antagonists are spotted onto porous chromatography paper and allowed to migrate through a portion previously impregnated with an antibody to the HA2. The aim of this method is to identify HA2-containing molecule-antagonist combinations which can no longer bind to the antibody. Identification of HA2-containing molecules whose migration is not retarded provides for a potential antagonist for binding to a  
20 porphyrin-containing molecule such as but not limited to hemoglobin or a precursor form thereof or part thereof such as heme. Antibody mAb 5A1 may be used where more conformational antagonists are sought in the general HA2 region.

There are many variations to the assays for screening for antagonists and all are  
25 encompassed by the present invention.

When the HA2-containing molecules or derivatives, analogues or homologues thereof are used in a vaccine composition, they are generally used as an immunogenic component to stimulate an immune response against their HA2 domain. They may  
30 also generate an immune response to other domains since this may cause conformational changes preventing HA2 interaction with a porphyrin-containing

molecule such as but not limited to hemoglobin or a precursor form thereof or part thereof such as heme.

Accordingly, another aspect of the present invention provides a composition such as  
5 therapeutic or vaccine composition comprising an agent as hereinbefore described and one or more pharmaceutically acceptable carriers and/or diluents.

The immunogenic component of a vaccine composition as contemplated herein exhibits excellent therapeutic activity, for example, in the prophylaxis and/or treatment  
10 of *P. gingivalis* infection when administered in an amount which depends on the particular case. For example, for recombinant peptide, polypeptide or protein molecules, from about 0.5  $\mu$ g to about 20 mg, may be administered, preferably from about 1  $\mu$ g to about 10 mg, more preferably from about 10  $\mu$ g to about 5 mg, and most preferably from about 50  $\mu$ g to about 1 mg equivalent of the immunogenic component  
15 in a volume of about 0.01ml to about 5 ml or from about 0.1ml to about 5 ml. The important feature is to administer sufficient immunogen to induce a protective immune response. The above amounts can be administered as stated or calculated per kilogram of body weight. Dosage regime can be adjusted to provide the optimum therapeutic response. For example, several divided doses can be administered or the  
20 dose can be proportionally reduced as indicated by the exigencies of the therapeutic situation. Booster administration may also be required.

The vaccine of the present invention can further comprise one or more additional immunomodulatory components such as, for example, an adjuvant or cytokine  
25 molecule, amongst others, which is capable of increasing the immune response against the immunogenic component. Non-limiting examples of adjuvants that can be used in the vaccine of the present invention include the RIBI adjuvant system (Ribi Inc., Hamilton, MT, USA), alum, mineral gels such as aluminium hydroxide gel, oil-in-water emulsions, water-in-oil emulsions such as, for example, Block co-polymer  
30 (CytRx, Atlanta GA, USA), QS-21 (Cambridge Biotech Inc., Cambridge MA, USA), SAF-M (Chiron, Emeryville CA, USA), AMPHIGEN<sup>®</sup> adjuvant, Freund's complete adjuvant;

Freund's incomplete adjuvant; and Saponin, QuilA or other saponin fraction, monophosphoryl lipid A, and Avridine lipid-amine adjuvant. Other immunomodulatory agents that can be included in the vaccine include, for example, one or more cytokines, such as interferon and/or interleukin, or other known cytokines. Non-ionic  
5 surfactants such as, for example, polyoxyethylene oleyl ether and n-hexadecyl polyethylene ether may also be included in the vaccines of the present invention.

The vaccine composition can be administered in any convenient manner such as by oral, intravenous (where water soluble), intramuscular, subcutaneous, intranasal,  
10 intradermal or suppository routes or by implantation (e.g., using slow release technology). Depending on the route of administration, the immunogenic component may be required to be coated in a material to protect it from the action of enzymes, acids and other natural conditions which may inactivate it, such as those in the digestive tract.

15

The vaccine composition may also be administered parenterally or intraperitoneally. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof, or in oils. Under ordinary conditions of storage and use, these preparations can contain a preservative to prevent the growth of microorganisms. Alternatively, the  
20 vaccine composition can be stored in lyophilised form to be rehydrated with an appropriate vehicle or carrier prior to use.

Pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous  
25 preparation of sterile injectable solutions or dispersions. In all cases the form must be fluid to the extent that easy syringeability exists, unless the pharmaceutical form is a solid or semi-solid such as when slow release technology is employed or it may be deliverable by spray, inhalation, nasal drip or microdroplets. In any event, it must be stable under the conditions of manufacture and storage and must be preserved  
30 against the contaminating action of microorganisms.

The carrier may be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance  
5 of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents such as, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases, it will be preferable to include isotonic agents such as, for example, sugars or sodium chloride. Prolonged  
10 absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption such as, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compound in the  
15 required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter-sterilization. Generally, dispersions are prepared by incorporating the sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients selected from those enumerated above. In the case of sterile powders for the  
20 preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

25 The present invention extends to vaccine compositions which confer protection against infection by one or more isolates or subtypes of *P. gingivalis* including those that belong to the same serovar or serogroup as *P. gingivalis*. The vaccine composition preferably also confers protection against infection by other species of the genus *Prophyromonas* or other microorganisms related thereto as determined at the  
30 nucleotide, biochemical, structural, physiological and/or immunointeractive level; the only requirement being that said other species or other microorganism produce a

peptide, polypeptide or protein which is immunologically cross-reactive to an HA2-containing molecule of *P. gingivalis*. For example, such related microorganisms may comprise genomic DNA which is at least about 70% similar overall to the genomic DNA of *P. gingivalis* as determined using standard genomic DNA hybridisation and  
5 analysis techniques.

The terms "serogroup" and "serovar" relate to a classification of microorganisms which is based upon serological typing data, in particular data obtained using agglutination assays such as the microscopic agglutination test (MAT). Those skilled in the art will  
10 be aware that serovar and serogroup antigens are a mosaic on the cell surface and, as a consequence there will be no strict delineation between bacteria belonging to a serovar and/or serogroup. Moreover, organisms which belong to different species may be classified into the same serovar or serogroup because they are indistinguishable by antigenic determination. As used herein, the term "serovar" means one or more *P.*  
15 *gingivalis* strains which are antigenically-identical with respect to antigenic determinants produced by one or more loci. Quantitatively, serovars may be differentiated from one another by cross-agglutination absorption techniques. As used herein, the term "serogroup" refers to a group of *Porphyromonas* spp. whose members cross-agglutinate with shared group antigens and do not cross-agglutinate with the  
20 members of other groups and, as a consequence, the members of a serogroup have more or less close antigenic relations with one another by simple cross-agglutination.

The present invention extends further to vaccine compositions capable of conferring protection against a "genetic variant" of *P. gingivalis*, the only requirement being that  
25 such a variant produce an HA2-containing peptide, polypeptide or protein capable of binding to an HA2-binding motif on a porphyrin-containing molecule. For example, the HA2-containing molecule may comprise at least about 50% similarity with respect to a *P. gingivalis* protein or at least about 25% identity after optimum alignment with same sequence.

30

The present invention further extends to combination vaccines comprising an effective

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amount of an immunogenic component of the present invention combined with an effective amount of one or more other antigens or therapeutic molecules capable of protecting the subject against other pathogens or disease conditions.

- 5 Particularly useful therapeutic compositions comprise antibodies to the porphyrin-binding motif of the HA2 domain or to other regions of the HA2 domain but which conformationally inhibits porphyrin binding.

The present invention further provides for the use of a gingipain or an HA2 domain  
10 containing part thereof or other HA2-containing molecule in the manufacture of a medicament for the prevention or treatment of infection by *P. gingivalis* or related microorganism.

In a related aspect of the present invention, there is provided a use of an antagonist  
15 of interaction between a HA2-containing molecule from *P. gingivalis* or related microorganism and a porphyrin-containing molecule such as, but not limited to hemoglobin or a precursor form thereof or part thereof such as heme in the manufacture of a medicament for the prophylaxis or treatment of *P. gingivalis* infection.



The present invention is further described by the following non-limiting Figures and Examples.

In the Figures:

5

**Figure 1** is a diagrammatic representation of the domain structure and homologies between the gingipains, RGP-1 and KGP. CAT represents putative catalytic domain and HA represents putative hemagglutinin domains. Shaded areas represent regions of > 98% amino acid identity between the two gingipains. Fractions represent the  
10 degree of the identity for each RGP-1 domain.

**Figure 2** is a graphical representation showing hemoglobin binding by rHA2, RGP-1, and KGP. 2a: Microtiter wells were coated with hemoglobin then incubated with 3 fold dilutions of purified rHA2, 2.5  $\mu\text{g/ml}$  (diamonds), RGP, 5  $\mu\text{g/ml}$  (circles), or KGP, 5  
15  $\mu\text{g/ml}$  (triangles). Association of rHA2 with hemoglobin was measured with mAb 5A1 followed by substrate development at 414 nm after binding of secondary anti-mouse alkaline phosphatase-conjugated antibody. 2b: Hemoglobin binding by native but not denatured gingipains. Wells were coated with hemoglobin then incubated overnight with dilutions of either RGP-1 (closed circles), KGP (closed triangles), or RGP-1  
20 denatured by boiling (open circles) or KGP denatured by boiling (open triangles). For this experiment, native or denatured gingipains that bound to hemoglobin were recognised with mAb IIB2, which specifically detects both native and denatured gingipains. Primary antibody IIB2 was followed by substrate development at 414 nm after binding of secondary anti-mouse AP-conjugated antibody. Data are  
25 representative of three separate experiments.

**Figure 3** is a graphical representation showing binding of the HA2 domain to the heme moiety. 3a: Binding of rHA2 to dilutions of hemin (diamonds), hemoglobin (circles), or hemoglobin degraded by proteinase-K (triangles). Microtiter wells were coated with  
30 dilutions of samples then overnight binding of rHA2 to coated wells was detected with mAb 5A1 followed by substrate development at 414 nm after binding of secondary

anti-mouse AP-conjugated antibody. The absence of contaminating protein within 90  $\mu$ g of the hemin preparation and the absence of non-degraded subunits of hemoglobin remaining after proteinase-K treatment was verified by SDS-PAGE (data not shown).

3b: Binding of rHA2 to hemin. Microtiter wells were coated with hemin and overnight binding of rHA2 dilutions was detected with mAb 5A1 as above. Data are representative of two separate experiments.

**Figure 4** is a graphical representation showing inhibition of hemin- or hemoglobin-binding. Microtiter wells were coated overnight with hemin (panel a ) or hemoglobin (panel b). rHA2 in *E. coli* lysate (100 fold dilution) (X), 65 ng/ml RGP-1 (circles) or 65ng/ml KGP (triangles) were preincubated with dilutions of 300  $\mu$ M protoporphyrin IX for 1 h then transferred to the ligand-coated plates for overnight incubation. Binding of rHA2 or the gingipains to coated wells was detected with mAb 5A1 or mAb IIB2, respectively, followed by substrate development at 414 nm after binding of secondary anti-mouse AP-conjugated antibody. Data are representative of two separate experiments. The absence of contaminating protein in a 90  $\mu$ g protoporphyrin IX preparation was verified by SDS-PAGE and by Coomassie dye binding.

**Figure 5** is a diagrammatic representation showing directed porphyrin-binding by rHA2. Microtiter wells were coated with 100 mM ethylene diamine (pH 4.7) then incubated with 90  $\mu$ g/ml hemin, protoporphyrin IX, or hematoporphyrin overnight in 50% dimethyl formamide in the presence (+) or absence (-) of 10 mM carbodiimide. Wells were washed 4 times with water then the amount of porphyrin bound to the wells was determined by absorbance at 414 nm (striped bars). Wells were blocked with PBS/Tween then incubated with 125 ng/ml rHA2 overnight. Binding of rHA2 to coated wells was detected with mAb 5A1 followed by substrate development at 414 nm after binding of secondary anti-mouse AP-conjugated antibody (solid bars). Error bars represent standard deviation of absorbance measurements. Diagrams of chemical structures for hemin, protoporphyrin IX, and hematoporphyrin are presented adjacent to corresponding data.

**Figure 6** is a graphical representation showing measurement of high-affinity binding of mAb 5A1 with rHA2 gingipains and gingipains from the culture supernatant. 6a: RGP-1 (circles), KGP (triangles) or rHA2 in crude *E. coli* lysate (squares) were coated onto microtiter wells and incubated with serial dilutions of mAb 5A1. 6b: Dilutions of RGP-1 (open circles), KGP (open triangles), or heat denatured RGP-1 (closed circles) or KGP (closed triangles) were coated onto microtiter wells with 3 fold dilutions from 10  $\mu$ g/ml then incubated with mAb 5A1. 6c: purified rHA2 (squares) or purified high molecular-weight aggregates of gingipain domains isolated from culture supernatant (circles) were coated onto microtiter wells and incubated with serial dilutions of mAb 5A1. Data are representative of three separate experiments.

**Figure 7** is a graphical representation showing immunoreactivity of synthetic peptides with mAb 5A1. ELISA demonstrating selective immunoreactivity of mAb 5A1 with peptide #1. Peptide #1 (squares) or peptide #2 (triangles) were coated onto microtiter plates at a concentration of 5  $\mu$ g/ml overnight then incubated with dilutions of mAb 5A1. Data are representative of two separate experiments.

**Figure 8** is a graphical representation showing expression of HA2-related immunoreactive hemoglobin-binding protein from *P. gingivalis*. Aliquots of *P. gingivalis* culture medium were removed daily during a period of 8 d and immediately separated into a cell pellet and culture supernatant then frozen until use. OD<sup>660</sup> and purity of the culture were measured daily. The cell pellets were dispersed evenly into 1 ml of PBS/N<sub>3</sub>. 8a and b: Arg- and Lys-specific proteinase activities, respectively, of the cell-free culture supernatant (squares) and cellular fraction (triangles) were measured as described. Measurements of the cellular fractions were normalised to culture densities (OD<sup>660</sup>) recorded daily. 8c: The HA2 domain (1/243 dilution, open squares) and HA2 domain associated with hemoglobin-binding (1/81 dilution, solid squares) in culture supernatants were measured by ELISA and ligand binding assay, respectively, as described. In *P. gingivalis* whole cell fractions, the HA2 domain (1/243 dilution, open triangles) and HA2 domain associated with hemoglobin-binding (1/9 dilution, solid triangles) were measured by ELISA and ligand binding assay, respectively, as

- 25 -

described. Measurements of the cell-associated fractions were normalised to culture densities ( $OD^{660}$ ) recorded daily. Corresponding background immunoreactivity with a murine anti-human CD-19 IgG was subtracted from each measurement. Data are representative of two separate experiments in which patterns of expression were similar.

TABLE 1

	Amino Acid	Three-letter Abbreviation	One-letter Symbol
5	Alanine	Ala	A
	Arginine	Arg	R
	Asparagine	Asn	N
	Aspartic acid	Asp	D
10	Cysteine	Cys	C
	Glutamine	Gln	Q
	Glutamic acid	Glu	E
	Glycine	Gly	G
	Histidine	His	H
15	Isoleucine	Ile	I
	Leucine	Leu	L
	Lysine	Lys	K
	Methionine	Met	M
	Phenylalanine	Phe	F
20	Proline	Pro	P
	Serine	Ser	S
	Threonine	Thr	T
	Tryptophan	Trp	W
	Tyrosine	Tyr	Y
25	Valine	Val	V
	Any residue	Xaa	X

**EXAMPLE 1****RGP-1 and KGP isolation**

Polydomain RGP-1 and KGP were isolated and characterised as described (18) by  
5 Arginine-sepharose affinity chromatography of detergent-extracted *P. gingivalis* (ATCC  
33277) cells. Alternatively, polydomain RGP-1 and KGP were isolated as previously  
described (19) by arginine-Sepharose affinity chromatography from cell-free  
supernatant of a 10 d *P. gingivalis* batch culture.

10

**EXAMPLE 2****Enzyme activity assays**

The proteinase activities of *P. gingivalis* culture fractions were measured using the  
substrates N-tertiary-butoxycarbonyl-Ile-Glu-Gly-Arg-7-amido-4-methylcoumarin or N-  
15 tertiary-butoxycarbonyl-Glu-Lys-Lys-7-amido-4-methylcoumarin at 30°C in Tris buffer  
without added reducing agents. Substrate hydrolysis was monitored over time by  
absorption at 460 nm using a 380 nm excitation beam on a Perkin Elmer LS 50B  
luminescence spectrophotometer.

20

**EXAMPLE 3****Development of monoclonal antibodies 5A1 and IIB2**

Anti-gingipain monoclonal antibodies 5A1 and IIB2 were prepared in mice against  
gingipains as described (19).

25

**EXAMPLE 4****Expression and purification of rHA2**

Forward and reverse primers (AACCTGCAGCGCGCAGACTTCACGG <400>2 and  
30 GGAAGCCAATGGCGCCAAAAGATCTAGT <400>3) were designed to amplify the  
HA2 domain from the *P. gingivalis* Arg-gingipain-1 proteinase gene (accession number

U15282). Restriction sites for *Pst*I and *Bgl*II were designed into the 5' ends of the primers to facilitate cloning. Digested PCR product was ligated into the QIAexpressionist type III construct providing a 6x-His tag on the COOH-terminus (Qiagen Corp., USA). Transformation of the ligated construct was performed by electroporation into *E. coli* NM522 cells. *E. coli* cultures were grown at 37°C to an OD<sup>600</sup> = 0.6 then induced with 0.1 mM isopropyl β-D-thiogalactopyranoside (IPTG) for 6 hours. Cells were harvested and resuspended to 5 ml per gram wet weight in buffer A (8 M Urea, 0.1 mM NaH<sub>2</sub>PO<sub>4</sub>, 0.01 mM Tris-HCl, pH 7.9). The cells were stirred for 2 h at room temperature taking care to avoid foaming. This cell lysate was subjected to centrifugation at 31k x g for 30 min at room temperature to pellet the cellular debris then the supernatant was subjected to ultracentrifugation at 130k x g for 2 h. The clarified lysate was loaded onto a nickel-nitrilotriacetic acid column (Ni-NTA, Qiagen Corp., USA) pre-equilibrated with buffer A. The Ni-NTA column was washed with buffer A until baseline was reached. The protein was refolded on this column by running a linear gradient of urea from 8 M to 0 M in 20 mM Tris-HCl, 500 mM NaCl, 10% v/v glycerol, pH 7.9. The protein was then eluted with 50 mM Tris-HCl, 500 mM NaCl, 10% v/v glycerol, 250 mM imidazole, pH 7.9. The eluant was diluted 100 fold in 50 mM sodium acetate buffer, pH 5.5 and applied to a hemoglobin-agarose column pre-equilibrated with the dilution buffer. After loading, the column was washed with the same buffer until baseline was reached then the hemoglobin-binding protein was eluted with 50 mM Tris-HCl, pH 9. Protein concentrations were determined by Coomassie dye binding using bovine serum albumin as a standard.

## EXAMPLE 5

25

### SDS-PAGE and Western blotting

SDS-PAGE was performed using 12% w/v gels with 4% w/v stackers by the method of Laemmli (39). All samples were diluted with SDS sample buffer before electrophoresis with (reducing) or without 2-mercaptoethanol. Western blots were performed by the method of Towbin (40) and proteins were transferred from the gels to polyvinylidene difluoride (PVDF) paper (BioRad Inc., CA, USA) with 300 mA for 1h. Blots

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were blocked with 0.1% w/v bovine serum albumin in 20 mM Tris-HCl with 500 mM NaCl containing 0.1% v/v Tween 20 (TBS/Tween). An alkaline phosphatase (AP) conjugate of a rabbit anti-mouse IgG (Dako Corp., USA) was used as a secondary antibody. Blots were washed with TBS/Tween between antibody applications.

5 Substrate for AP was nitroblue tetrazolium in excess with 5-bromo-4-chloro-3-indoyl phosphate (NBT/BCIP) (BioRad, CA, USA) and color was developed in 5 mM Tris, pH 9.5.

NH<sub>2</sub>-terminal amino acid sequencing of resolved SDS-PAGE proteins was performed

10 as previously described (41).

## EXAMPLE 6

### ELISA

15 Enzyme linked immunosorbant assays (ELISA) were performed in polystyrene microtiter wells. Proteins were coated onto the surfaces in 2.7 mM KCl, 1.5 mM KH<sub>2</sub>PO<sub>4</sub>, 137 mM NaCl, 8.1 mM Na<sub>2</sub>HPO<sub>4</sub> (PBS) with 10 mM sodium azide (PBS/N<sub>3</sub>). All wells were blocked and washed in PBS with 0.1% v/v Tween 20 (PBS/Tween). Primary murine antibodies were applied in PBS/Tween at a concentration of 0.5 µg/ml

20 for at least 1 h. Secondary goat anti-mouse antibodies conjugated with AP (Dako Corp., USA) were applied at a concentration of 1.1 µg/ml for 30 min then AP activity was monitored at 414 nm by hydrolysis of the substrate 4-Nitrophenylphosphate (Boehringer Mannheim, Germany) in 5 mM Tris, pH 9.5 using a Titertek Twinreader PLUS photometer (absorbance maximum of 3.0 ELISA units). Apparent dissociation

25 constants ( $K_d$ ) were derived by solid-phase with ELISA as previously described (42) and are accompanied by standard error of the means.



- 30 -

## EXAMPLE 7

### Ligand binding assay

The ligand binding assay was a variant of the ELISA in which the ligand (ie. hemin or  
5 hemoglobin) that had been coated onto the wells in PBS/N<sub>3</sub> was subsequently allowed  
to bind to a second ligand binding protein (ie. rHA2 or gingipains) in PBS/Tween. The  
ligand binding protein was then detected with mAb 5A1 or mAb IIB2 which was  
followed by a rabbit anti-mouse AP conjugate and developed as described for ELISA.  
Bovine hemoglobin was used in these experiments. Hemin was from stock solutions  
10 dissolved in 0.1 N NaOH and, although the NaOH would replace the chloride ion of  
hemin with an hydroxylate ion (hematin), the term hemin is used herein for this  
compound.  $K_d$  and apparent inhibition constants ( $K_i$ ) for ligand binding were derived  
as previously described (42) in these assays using serial dilutions of ligand binding  
protein or competitor, respectively, with even amounts of coated ligand. Results are  
15 accompanied by standard error of the means.

## EXAMPLE 8

### Peptide synthesis

20 Peptides were synthesised by Chiron Mimotopes (Victoria, Australia) with terminal  
amines and carboxylic acids. Peptide #1 sequence was ALNPDNYLISKDVTG  
<400>1. Peptide #2 sequence was GEAPAEWTTIDADGDGQGWL <400>4.

## EXAMPLE 9

25

### Materials

All chemicals and compounds were purchased from Sigma Corp., NSW, Australia  
unless otherwise herein specified.

**EXAMPLE 10****Statistics**

Statistical differences of measurements between the gingipains and rHA2 were  
 5 determined with one-tailed Student's t-tests.

**EXAMPLE 11****Characteristics of RGP-1 and KGP**

- 10 The polydomain Lys-and Arg-gingipains (RGP-1 and KGP, respectively) isolated from the CHAPS-extracted *P. gingivalis* cells possessed SDS-PAGE profiles, NH<sub>2</sub>-terminal sequences, proteolytic activities and inhibition profiles characteristic of gingipain-like molecules previously described (19, 34).
- 15 The HA2 domain was cloned, expressed, and purified as a 6x His-tag fusion. Nucleic acid and NH<sub>2</sub>-terminal amino acid sequencing verified the identity of the clone and the expressed protein, respectively, as the HA2 domain of RGP-1.

**EXAMPLE 12**

- 20 **Hemoglobin is bound by rHA2 and by native but  
 not denatured RGP-1 and KGP**

Using the solid-phase ligand binding assay, rHA2, RGP-1, and KGP each bound to hemoglobin (Fig. 2a). As mAb 5A1 was used to detect rHA2 bound to hemoglobin and  
 25 did not interfere with this binding, it was evident that the epitope for mAb 5A1 within the HA2 domain was separate from the hemoglobin-binding site of HA2. Hemoglobin binding affinities were similar ( $P = .24$ ) for the rHA2, RGP-1 and KGP ( $K_d = 2.1 \pm 0.6$  nM) and the binding curves of neither the rHA2 nor the gingipains were indicative of multi-site binding (Fig. 2a). High-affinity binding to hemoglobin at a single site within  
 30 only the HA2 domain of both native RGP-1 and KGP is sufficient to account for these observations. The binding site for hemoglobin within the gingipains appeared to be

associated with higher-order protein structure since denaturation of RGP-1 and KGP by boiling effectively eliminated their ability to bind hemoglobin (Fig. 2b).

### EXAMPLE 13

#### 5 Hemoglobin binding of the HA2 domain is mediated through the heme moiety

To begin characterising the binding between rHA2 and hemoglobin, the inventors examined the binding between rHA2 and hemin as well as binding to hemoglobin degraded by proteinase-K. The rHA2 bound not only to wells coated with hemoglobin but also to wells coated with hemin or with the proteolytically degraded hemoglobin (Fig. 3a). Binding of the rHA2 domain to hemin-coated wells was approximately 8 fold weaker than binding to hemoglobin in solid-phase assays ( $K_d = 1.6 \pm 0.1 \times 10^{-8}$  M) (Fig. 3b).

15

### EXAMPLE 14

#### HA2 domain binds the porphyrin ring structure

To dissect the binding of the rHA2 domain to hemin, inhibition constants ( $K_i$ ) of the iron-free protoporphyrin IX in solution-phase competition assays were determined. Using the standard ligand binding assay described herein, rHA2 or the gingipains were preincubated with dilutions of protoporphyrin IX then allowed to bind to the hemin-coated wells. Binding of the gingipains or rHA2 to hemin was inhibited with the addition of protoporphyrin IX ( $K_i = 2.5 \pm 0.3 \mu\text{M}$ ) (Fig. 4a). Apparent  $K_i$  values were similar between rHA2 and gingipains ( $P = .42$ ). These data indicated that binding of rHA2 or the gingipains to hemin was specific for some aspect of the protoporphyrin ring. Importantly, binding of rHA2 or the gingipains to hemoglobin was also inhibited with protoporphyrin IX (Fig. 4b) ( $K_i = 10 \pm 2 \mu\text{M}$ ) and pre-incubation with the protoporphyrin could effectively eliminate binding to hemoglobin.

30

**EXAMPLE 15****Directed protoporphyrin binding by recombinant HA2 (rHA2)**

Examination of the hemoglobin crystal structure indicated that only the region of the heme moiety possessing the propionate functional groups (Fig. 5) would be exposed for possible protein/protein contact. The inventors reasoned, therefore, that blocking access to the acidic region of protoporphyrin molecules would have an effect on rHA2-binding and allow more specific characterisation of binding between the HA2 domain and the porphyrin ring. Modifying the ligand binding assay system used above, surfaces were first coated with ethylene diamine to provide fixed, free, primary amines for carbodiimide linkage of carboxylic acid groups. Hemin, protoporphyrin IX, and hematoporphyrin bound to wells coated with ethylene diamine with or without carbodiimide treatment as determined by absorbance at 414 nm (Fig. 5, striped bars). rHA2-binding to the carbodiimide-treated porphyrins in the wells was almost eliminated, however, compared to the relatively greater association of the rHA2 with the non-derivatised porphyrins (Fig. 5, solid bars). These data indicated that the rHA2 domain specifically recognised the three porphyrin compounds in the region of the propionic acid groups, as we were able to block rHA2-binding by directionally attaching the carboxylic acids of hemin, protoporphyrin IX, or hematoporphyrin to fixed amines. Since the heme moiety within hemoglobin is almost identical to these porphyrin molecules, the data suggested that the heme moiety of hemoglobin was bound by rHA2 and by the HA2 domain of the gingipains in a similar, directed, high-affinity manner.

**EXAMPLE 16**

**Epitope of mAb 5A1 is recognised in recombinant HA2 domain  
and in RGP-1 and KGP**

In ELISA, mAb 5A1 bound to the rHA2 with a high affinity ( $K_d = 2.2 \pm 0.5 \times 10^{-10}$  M) (Fig. 6a). mAb 5A1 also bound to RGP-1 and KGP isolated from the CHAPS-extracted *P. gingivalis* cells (Fig. 6b). Soluble high molecular-weight aggregates of gingipain

domains isolated from the cell-free fraction of a *P. gingivalis* batch culture by arginine-Sepharose affinity chromatography (34) were, however, recognised by mAb 5A1 ( $K_d = 1.7 \pm 0.6 \times 10^{-10}$  M) (Fig. 6c). Similarity of dissociation constants ( $P = .36$ ) and of the binding curves suggested that mAb 5A1 recognised the same HA2 epitope in these polydomain gingipains as in rHA2.

### EXAMPLE 17

#### Epitope of mAb 5A1 is represented by an amino acid sequence within the HA2 gingipain domain

10

Using linear synthetic peptides, the epitope of mAb 5A1 was determined to be associated with the peptide ALNPDNYLISKDVTG <400>1 ( $K_d = 3.8$  nM) which represents amino acids #1215-1229 of the translated KGP within the HA2 domain (Fig. 7, peptide #1). Dot blot analysis on PVDF membrane confirmed the unique immunoreactivity of this peptide with mAb 5A1. A search of SwissProt database for the linear sequence of peptide #1 or GenBank database using the deduced nucleic acid sequence of this epitope resulted in no molecules with perfect homology to the peptide other than the gingipains and HagA, a large hemagglutinin with regions of identity to the entire HA2 domain.

20

### EXAMPLE 18

#### Correlation of HA2 domain immunoreactivity with hemoglobin binding in *P. gingivalis* culture

25 Detection of the HA2 epitope with mAb 5A1 in unfractionated *P. gingivalis* samples was correlated with hemoglobin binding. Because proteinase activity and gingipain expression have been shown to progressively change during the course of an extended *P. gingivalis* batch culture (34), the inventors examined cell-associated and extra-cellular fractions during 8 days of culture. Both Arg- and Lys-specific proteinase activities of the *P. gingivalis* cells peaked near the third day of culture (Fig. 8a and b, triangles). Proteinase activities of the cell-free culture supernatants steadily rose

30

throughout the culture period (Fig. 8a and b, squares).

Immunoreactive protein in the cell-free conditioned culture medium detected with mAb 5A1 steadily accumulated throughout the 8 day culture period similar to proteolytic activity (Fig. 8c, open squares). Immunoreactive protein associated with hemoglobin-binding in this supernatant fraction also increased steadily throughout the extended culture in a parallel manner (Fig. 8c, closed squares). In the cellular fraction of the *P. gingivalis* culture, expression of immunoreactive protein increased early during the culture period with a peak near day 3 followed by a slight decrease then an increase to peak levels again by day 7, similar to proteolytic activity of this fraction (Fig. 8c, open triangles). Immunoreactive protein associated with hemoglobin-binding in the cellular fraction followed a parallel pattern of expression (Fig. 8c, closed triangles). These data demonstrated that detection of protein immunoreactive with mAb 5A1 in crude cellular and extra-cellular fractions of *P. gingivalis* culture was directly associated with hemoglobin-binding suggesting that mAb 5A1 specifically recognised the hemoglobin-binding, HA2 domain within *P. gingivalis* culture. Also, the data demonstrated a profile of HA2 domain expression and hemoglobin-binding activity similar with profiles of cellular and extra-cellular proteolytic activity expressed by *P. gingivalis*.

20

## EXAMPLE 19

### Controlling Porphyromonas gingivalis growth

The control of *P. gingivalis* growth with prevention of periodontal pathology is achieved by interfering one or more pathways for obtaining heme. To this end, the inventors have shown that a monoclonal antibody recognises an epitope within the hemoglobin-binding domain of the abundant *P. gingivalis* cysteine proteinases, i.e. gingipains, and have demonstrated increasing levels of this HA2 domain associated with hemoglobin-binding and proteinase activity in extended *P. gingivalis* culture. Further, the inventors have characterised the binding between the HA2 domain and hemoglobin suggesting that binding is mediated in large part through a specific recognition of the porphyrin

ring of the heme moiety within the hemoglobin.

Characterisation of the binding between the recombinant HA2 (rHA2) domain and porphyrins allows for the design of efficient affinity ligands for purifying HA2, and allow  
 5 structure-based design for heme/hemoglobin binding inhibitors. Heme acquisition is considered fundamental to the growth of *P. gingivalis*, and intervention with specific agents to disrupt pathways for heme-binding or uptake allows the control or prevention of periodontal disease.

10

#### EXAMPLE 20

##### ***Porphyromonas gingivalis* does not contain a functional porphyrin-biosynthetic pathway**

The presence or absence of genetic sequences encoding enzymes required for  
 15 porphyrin biosynthesis was assessed in *P. gingivalis*.

All entries for proteins used in the porphyrin biosynthesis pathway that are listed in the Swiss-Prot database (Release 37.0) were searched with TBlastN against the available genomic data for the *P. gingivalis* genome (45). This search comprised 257  
 20 eukaryotic, archaeal and prokaryotic protein sequences used in different pathways related to porphyrin biosynthesis. These are the standard enzymes in porphyrin biosynthesis, alternative enzymes used by subsets of organisms, the enzymes of the C<sub>4</sub> and C<sub>5</sub> pathways for 5-aminolevulinic acid synthesis, and enzymes used in Vitamin B<sub>12</sub> synthesis. Preliminary sequence data for *P. gingivalis* was obtained from The  
 25 Institute for Genomic Research through the website at <http://www.tigr.org>.

Open reading frames with some identity to enzymes required for heme synthesis were further examined by comparison with enzymes from 4 reference organisms: *Escherichia coli*, *Bacillus subtilis*, *Synechocystis* and *Aquifex aeolicus*, and the enzyme  
 30 5-aminolevulinic acid synthase from *Bradyrhizobium japonicum*. Open reading frames were identified using the program Map and translated with Translate (46). Sequence

identity between protein sequences was calculated using the program GAP and multiple alignments performed with PileUp (46).

Enzymes used solely in the pathway for vitamin B<sub>12</sub> synthesis were reported as a  
5 match if there was an open reading frame (ORF) which has a BLAST score greater than 100. Enzymes and proteins involved in cytochrome synthesis were also BLAST searched against the genome. Proteins were aligned and identity calculated using GAP (46).

10 The proteins identified in Table 2 were BLAST searched against the *P. gingivalis* genome. No significant matches were detected for the proteins glutamyl-tRNA reductase, porphobilinogen synthase, porphobilinogen deaminase, uroporphyrinogen III cosynthase, uroporphyrinogen decarboxylase, coproporphyrinogen III oxidase, HemM or uroporphyrinogen III methylase.

15

The above observations indicate that *P. gingivalis* is unable to undergo *de novo* synthesis of porphyrin. Accordingly, the early and essential steps for synthesis of the tetrapyrrole ring are not encoded in the genome of *P. gingivalis*. As this organism has several proteins associated with tetrapyrrole rings, it is concluded that *P. gingivalis* has  
20 a requirement for porphyrin.



**TABLE 2**  
**ENZYMES USED IN HEME BIOSYNTHESIS**

	<b>Enzyme</b>	<b>Function</b>
5	Hem1/Hem0/HemA	5-Aminolevulinic acid synthase
	HemA/Hem1	Glutamyl-tRNA reductase
	HemB/Hem2	Porphobilinogen synthase
	HemC/Hem3	Porphobilinogen deaminase
	HemD/CysG/NasF	Uroporphyrinogen III cosynthase
10	HemE/DcuP	Uroporphyrinogen decarboxylase
	HemF/Hem6	Coprotoporphyrinogen III oxidase
	HemH/HemZ	Ferrochelatase
	HemL/HemK	Glutamate-1-semialdehyde 2, 1 aminotransferase
	HemM	An enzyme in main pathway of synthesis of 5-aminolevulinate, possibly glutamyl-tRNA dehydrogenase
15	HemN	Oxygen-independent coproporphyrinogen III oxidase
	HemX	Uroporphyrinogen III methylase
	HemX	Protoporphyrinogen oxidase
	GltX	Glutamyl-tRNA synthetase

## EXAMPLE 21

### Clinical studies

Patients presenting to a dental hospital were selected for a clinical study. The  
 5 inclusion criteria were some level of adult periodontitis, no profession periodontal  
 treatment within the prior 3 years nor use of antibiotics within the prior 6 months. Two  
 donor sites, one relatively healthy and one with relatively advanced periodontal  
 disease were selected in each mouth on the basis of radiographic examination.  
 Clinical parameters were measured and plaque samples were obtained from each site.  
 10 All periodontal samples and measurements were obtained by one of the inventors.  
 Venous blood was collected within 30 min after plaque samples were obtained.  
 Differences between categories were established by Student's 2-tailed t-test using  
 95% confidence levels. The relationships of values between categories were  
 established by linear regression with a 95% confidence level. Levels of periodontal  
 15 disease severity were assigned the following values: 0, none; 1, localised mild; 2,  
 generalised mild; 3, localised moderate; 4, generalised moderate; 5, localised severe;  
 6, generalised severe.

Detection of the denatured HA2 domain with mAb 5A1 in plaque samples were more  
 20 frequent in the relatively diseased donor sites than in the healthier donor sites  
 ( $P=.004$ ) and detection of the HA2-associated epitope was positively associated with  
 levels of attachment loss at the corresponding site ( $P=.012$ ). The detection of HA2-  
 associated epitope in denatured plaque with mAb 5A1 was also significantly correlated  
 with the detection of haemoglobin-binding activity in plaque from the same sites  
 25 ( $P=.001$ ).

All of the patients had serum antibody recognising rHA2, RGP-1 and KGP. The  
 average titre for IgG antibody against RgpA was no different to IgG titre against KGP  
 and was 36 fold higher than for IgG antibody recognising the rHA2 domain in this  
 30 patient population ( $P<.001$ ). IgG fractions from sera with higher titres against the  
 purified gingipains were more effective in neutralising gingipain Arg- and Lys-specific

activities (RGP-1,  $P=.017$ ; KGP,  $P=.041$ ). Neutralisation of proteinase activity with IgG from pre-treatment sera did not, however, significantly correlate with clinical disease parameters. Neutralisation of haemoglobin-binding and proteinase activity was confirmed to be IgG dependent by removing the neutralising capacity of IgG fractions  
5 with a second pass over a protein-G affinity column. These data suggested that development of higher IgG titres to the gingipains may be associated with an increased functional antibody response in terms of the cysteine proteinase activities.

Sera collected some time after the commencement of periodontal therapy were also  
10 examined because of the possible inoculating effect that periodontal therapy might have on the host immune system. The commencement of treatment was significantly associated with an increase in IgG titre against the HA2 domain ( $P=.017$ ) with no change in the IgM or IgA titres. After the initiation of periodontal therapy, capacity for neutralisation of haemoglobin-binding of the gingipains with IgG from post-treatment  
15 sera had become negatively associated with the diagnosis of periodontal disease severity (RGP-1,  $P=.033$ ; KGP,  $P=.032$ ). These data suggested that development of higher IgG titres to the HA2 domain of the gingipains may be associated with an increased functional antibody response in terms of neutralisation of haemoglobin-binding activity.

20

These findings indicate that a functional immune response can be developed against the HA2 domain and neutralizing the cysteine proteinases from *P. gingivalis*. Inhibition of gingipain cysteine proteinase activity by serum IgG fractions was demonstrated to be associated with higher serum titres to the gingipains.

25

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds  
30 referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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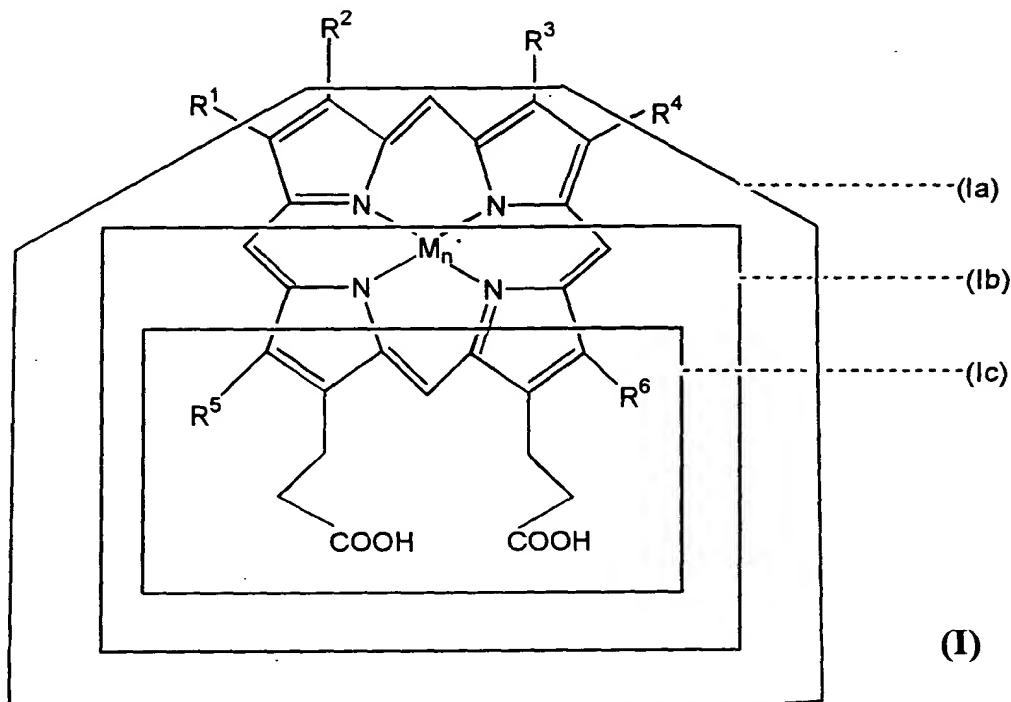
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VARIOUS NON-LIMITING ASPECTS OF THE PRESENT INVENTION ARE AS FOLLOWS:

1. A method for the prophylaxis or treatment of infection by a microorganism in a biological environment from where the microorganism acquires iron, heme or porphyrin said method comprising administering to said environment an effective amount of an agent for a time and under conditions sufficient to antagonise the interaction between a molecule derived from said microorganism and having an HA2 domain and an HA2-binding motif on a porphyrin containing molecule present in said biological environment.
2. A method according to aspect 1 wherein the microorganism is *Porphyromonas gingivalis* or a related microorganism.
3. A method according to aspect 2 wherein the biological environment is a mammal.
4. A method according to aspect 3 wherein the mammal is a primate, human, livestock animal or a companion animal.
5. A method according to any one of aspects 1 to 4 when used for the treatment of a disease condition in the oral cavity, nasopharynx, oropharynx, vagina or urethra or other vascular or mucosal regions or cavities or in the hooves of livestock animals.
6. A method according to any one of aspects 1 to 5 wherein the HA2-containing molecule is a gingipain, an hagA gene product or a TonB-dependent protein such as but not limited to Tla protein or a homologue thereof.
7. A method according to aspect 1 or 6 wherein the porphyrin moiety is heme.

8. A method according to aspect 7 wherein the HA2-binding motif comprises a region comprising or within substructure (Ia) of structure (I):



9. A method for the prophylaxis or treatment of infection by a microorganism in a mammal, said microorganism substantially requiring exogenous iron, heme or porphyrin for growth or maintenance wherein said method comprises administering to said mammal an effective amount of an agent for a time and under conditions sufficient to antagonise the interaction between a molecule derived from said microorganism and having an HA2 domain and an HA2-binding moiety on a porphyrin-containing molecule such as but not limited to hemoglobin or a precursor form thereof or part thereof such as heme and wherein said HA2 domain comprises:

(i) an amino acid sequence substantially encoded by the nucleotide sequence set forth in <400>5 or a nucleotide sequence having at least about 50% similarity thereto or capable of hybridizing thereto under low stringency conditions; and/or

(ii) an amino acid sequence substantially as set forth in <400>6 or an amino acid sequence having at least about 50% similarity thereto or at least about 25%

identity after optimum alignment with same sequence.;

wherein said amino acid sequence is capable of interacting with an HA2-binding moiety on a porphyrin-containing molecule such as but not limited to hemoglobin or a precursor form thereof or part thereof such as heme.

10. A method for prophylaxis or treatment of periodontal, pulmonary, vaginal, urethral or hoof disease resulting from infection by *P. gingivalis* or related microorganism in a mammal said method comprising administering to said mammal an effective amount of a agent for a time and under conditions sufficient to antagonise the interaction between a *P. gingivalis*-derived molecule having an HA2 domain and an HA2-binding motif on hemoglobin.

11. An agent capable of antagonising interaction between an HA2-containing molecule and an HA2-binding motif on a porphyrin-containing molecule such as but not limited to hemoglobin or a precursor form thereof or part thereof such as heme.

12. An agent according to aspect 11 wherein the porphyrin is heme.

13. Use of a gingipain or an HA2 domain containing part thereof or an HA2-containing molecule in the manufacture of a medicament for the prevention or treatment of *P. gingivalis* infection or infection by a related microorganism.

14. Use of an antagonist of *P. gingivalis*-derived HA2-containing molecule interaction with a porphyrin-containing molecule such as but not limited to hemoglobin or a precursor form thereof or part thereof such as heme in the manufacture of a medicament for the prophylaxis or treatment of *P. gingivalis* infection or infection by a related microorganism.

15. A therapeutic composition comprising an agent according to any one of aspects 11 to 12 and one or more pharmaceutically acceptable carriers and/or diluents.

- 1 -

SEQUENCE LISTING

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<120> A METHOD OF PROPHYLAXIS AND TREATMENT AND AGENTS USEFUL  
FOR SAME

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&lt;400&gt; 5

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gcg gaa tgg act act atc gat gcc gat ggc gat ggt gag ggt tgg ctc				96
Ala Glu Trp Thr Thr Ile Asp Ala Asp Gly Asp Gly Glu Gly Trp Leu				
	20	25	30	
tgt ctg tct tcc gga caa ttg gac tgg ctc aca gct cat ggc ggc acc				144
Cys Leu Ser Ser Gly Gln Leu Asp Trp Leu Thr Ala His Gly Gly Thr				
	35	40	45	
aac gta gta agc tct ttc tca tgg aat gga atg gct ttg aat cct gat				192
Asn Val Val Ser Ser Phe Ser Trp Asn Gly Met Ala Leu Asn Pro Asp				
	50	55	60	
aac tat ctc atc tca aag gat gtt aca ggc gca acg aag gta aag tac				240
Asn Tyr Leu Ile Ser Lys Asp Val Thr Gly Ala Thr Lys Val Lys Tyr				
	65	70	75	80
tac tat cca gtc aac gac ggt ttt ccc ggg gat cac tat gcg gtg atg				288
Tyr Tyr Pro Val Asn Asp Gly Phe Pro Gly Asp His Tyr Ala Val Met				
	85	90	95	
atc tcc aag acg ggc acg aac gcc gga gac ttc acg gtt gtt ttc gaa				336
Ile Ser Lys Thr Gly Thr Asn Ala Gly Asp Phe Thr Val Val Phe Glu				
	100	105	110	
gaa acg cct aac gga ata aat aag ggc gga gca aga ttc ggt ctt tcc				384
Glu Thr Pro Asn Gly Ile Asn Lys Gly Gly Ala Arg Phe Gly Leu Ser				
	115	120	125	
acg gaa gcc aat ggc gcc				402
Thr Glu Ala Asn Gly Ala				
	130			

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- 4 -

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Cys Leu Ser Ser Gly Gln Leu Asp Trp Leu Thr Ala His Gly Gly Thr  
 35 40 45

Asn Val Val Ser Ser Phe Ser Trp Asn Gly Met Ala Leu Asn Pro Asp  
 50 55 60

Asn Tyr Leu Ile Ser Lys Asp Val Thr Gly Ala Thr Lys Val Lys Tyr  
 65 70 75 80

Tyr Tyr Pro Val Asn Asp Gly Phe Pro Gly Asp His Tyr Ala Val Met  
 85 90 95

Ile Ser Lys Thr Gly Thr Asn Ala Gly Asp Phe Thr Val Val Phe Glu  
 100 105 110

Glu Thr Pro Asn Gly Ile Asn Lys Gly Gly Ala Arg Phe Gly Leu Ser  
 115 120 125

Thr Glu Ala Asn Gly Ala  
 130

&lt;210&gt; 7

&lt;211&gt; 63

&lt;212&gt; DNA

&lt;213&gt; Porphyromonas gingivalis

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1) .. (63)



- 5 -

&lt;400&gt; 7

gct ttg aat cct gat aac tat ctc atc tca aag gat gtt aca ggc gca 48  
 Ala Leu Asn Pro Asp Asn Tyr Leu Ile Ser Lys Asp Val Thr Gly Ala  
 1 5 10 15

acg aag gta aag tac 63  
 Thr Lys Val Lys Tyr  
 20

&lt;210&gt; 8

&lt;211&gt; 21

&lt;212&gt; PRT

&lt;213&gt; Porphyromonas gingivalis

&lt;400&gt; 8

Ala Leu Asn Pro Asp Asn Tyr Leu Ile Ser Lys Asp Val Thr Gly Ala  
 1 5 10 15

Thr Lys Val Lys Tyr  
 20

DATED this 28th day of May, 1999

University of Sydney

By DAVIES COLLISON CAVE

Patent Attorneys for the Applicant

<b>RGP-1</b>	CAT	HA1	HA2	HA3	HA4	1476 amino acids
Approx. kDa	48	45	15	17	27	
<b>KGP</b>	CAT	HA1	HA2	HA3	HA4	1495 amino acids
Approx. kDa	52	40	15	17	27	
	24/491					

FIGURE 1

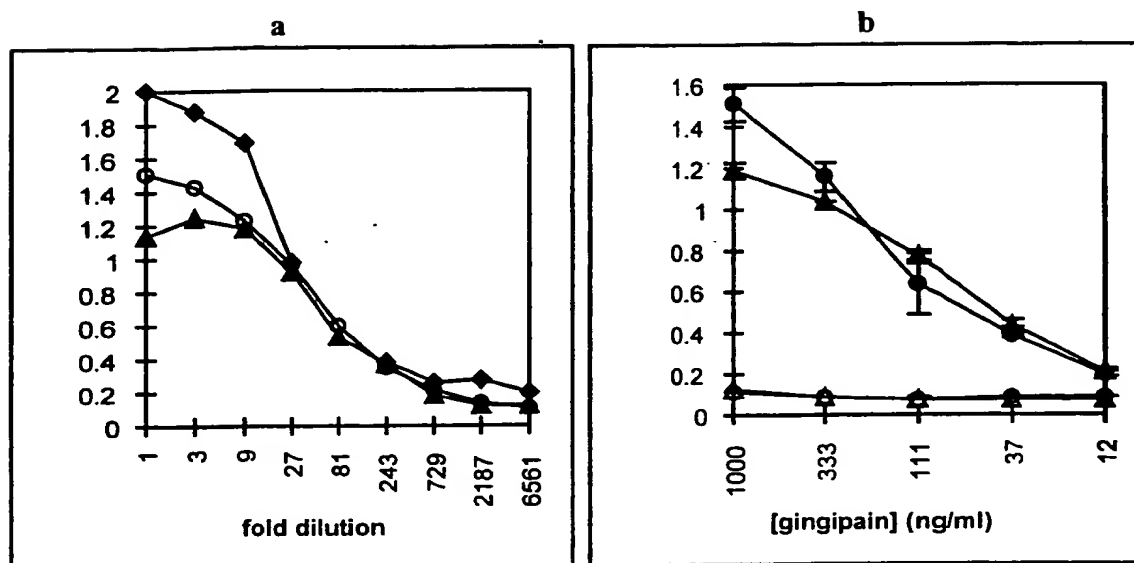


FIGURE 2

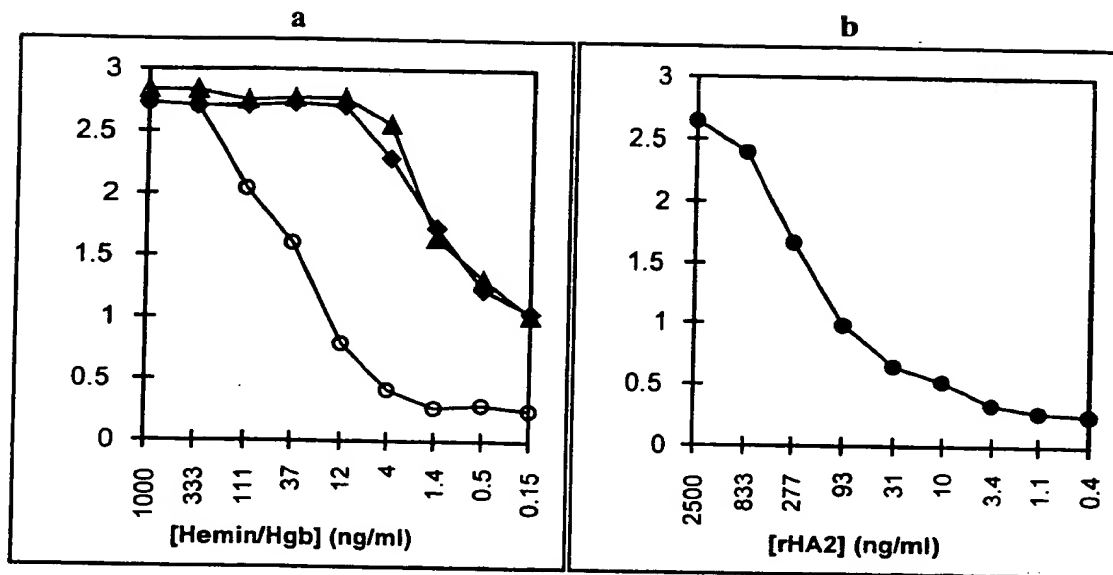


FIGURE 3

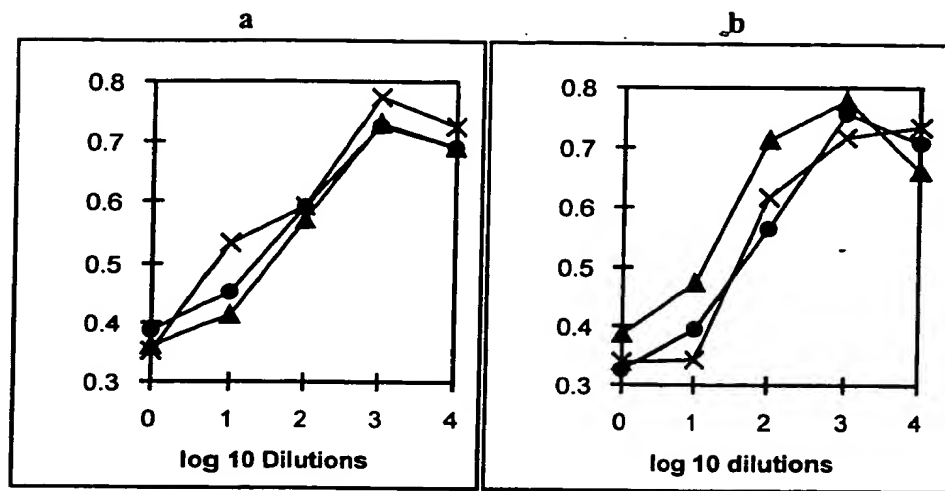
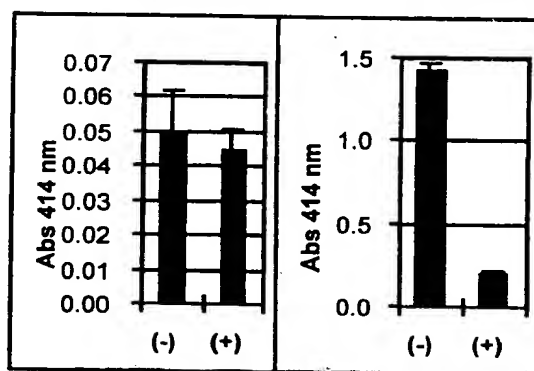
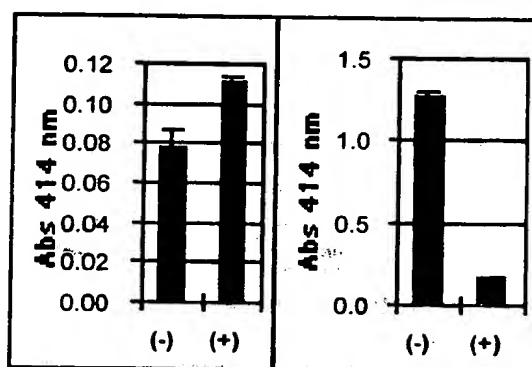
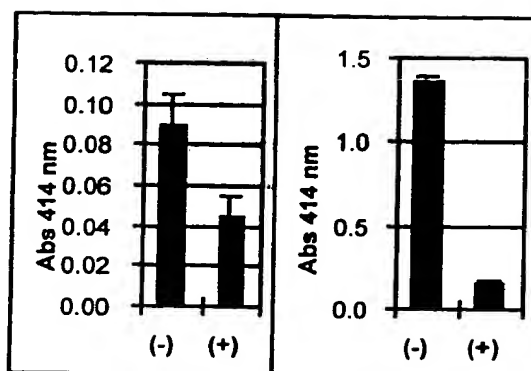


FIGURE 4

The chemical structure shows a central iron atom (Fe) coordinated by four nitrogen atoms (N) in a porphyrin-like ring. The ring is substituted with various side chains: a methyl group (H<sub>3</sub>C) and a vinyl group (CH=CH<sub>2</sub>) on the top-left pyrrole ring; a methyl group (CH<sub>3</sub>) and a vinyl group (CH=CH<sub>2</sub>) on the top-right pyrrole ring; a methyl group (H<sub>3</sub>C) and a propionate group (CH<sub>2</sub>CH<sub>2</sub>COOH) on the bottom-left pyrrole ring; and a methyl group (CH<sub>3</sub>) and a propionate group (CH<sub>2</sub>CH<sub>2</sub>COOH) on the bottom-right pyrrole ring. A hydroxymethyl group (H<sub>2</sub>C-OH) is attached to the top-left pyrrole ring.



## FIGURE 5

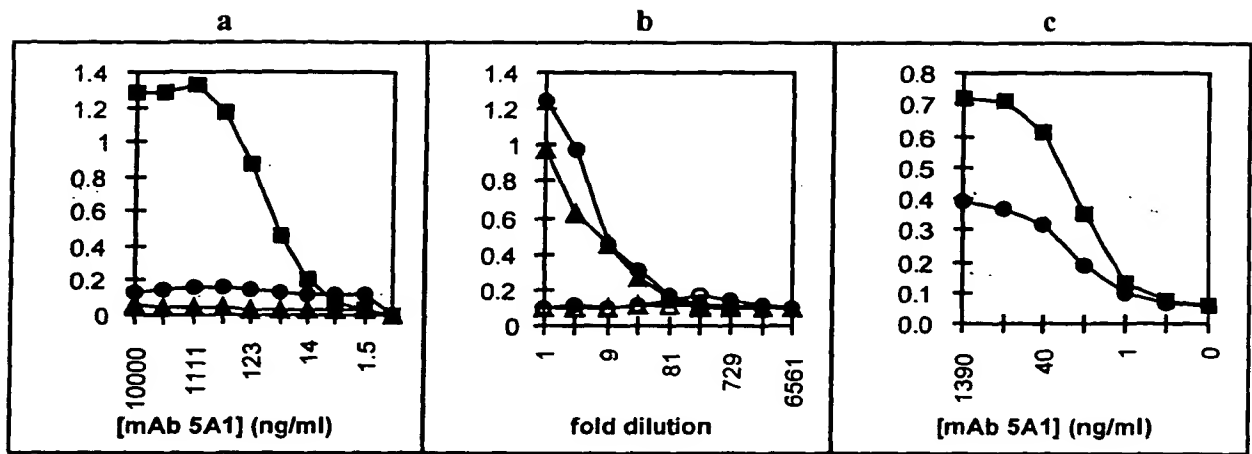


FIGURE 6

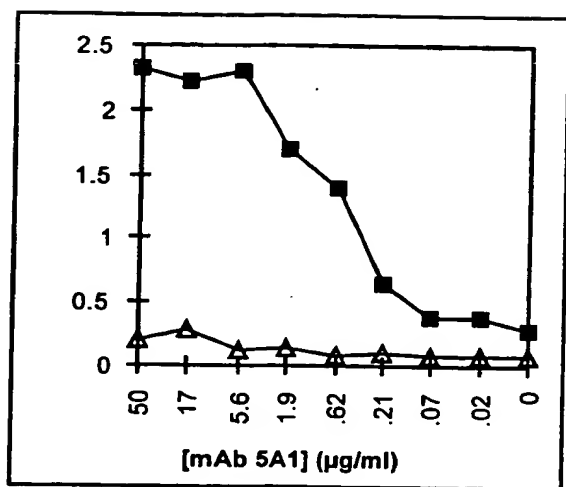


FIGURE 7



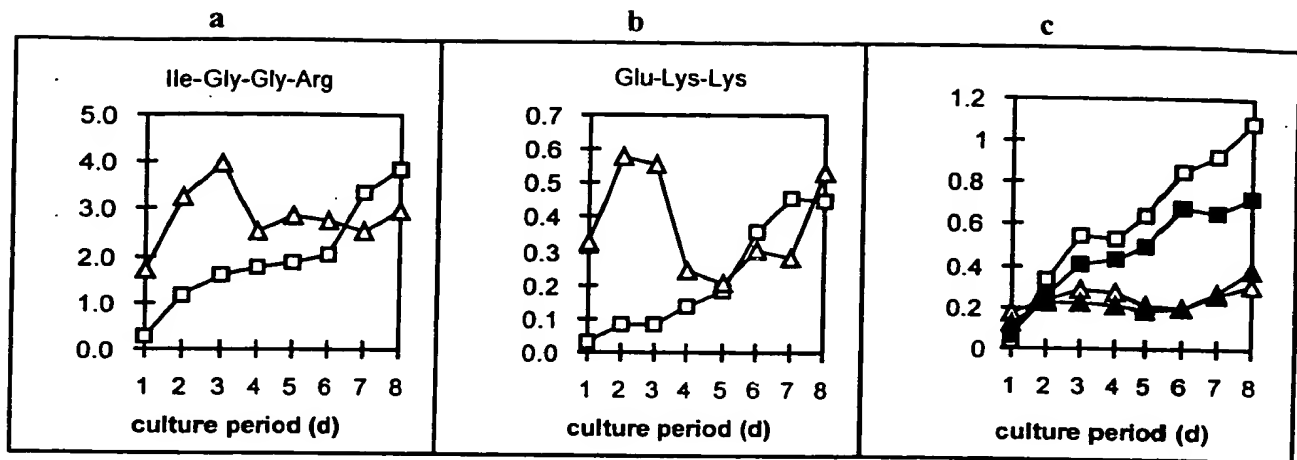


FIGURE 8

